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Effect of Common Household Insecticides Used in Nigeria on Rat Male Reproductive Hormones

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Authors' contributions

This work was carried out in collaboration among all authors. Author AIA conceptualized and designed the study and also wrote the manuscript. Author KON managed the analyses of the study and the literature searches. Author ACN wrote the protocol while author JAE performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Background: The use of household insecticides for the eradication of insects especially mosquitoes in Nigeria is increasing. These insecticides are used without consideration of their adverse effect on human health.
Aim: This study was aimed at accessing the effect of common household insecticides used in Nigeria on male reproductive hormones of Wistar rats.
Methodology: Thirty male Wistar rats were divided into five groups of six each and kept in different rooms. Rats in group 1 were exposed to Rambo, those in group 2 were exposed to Mortein, those in group 3 were exposed to Raid, those in group 4 were exposed to Baygon while those in group 5 were not exposed to any insecticide and served as the control group. The exposure was done twice a day via inhalation route. Throughout the experiment, animals were fed *ad libitum* with standard

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feed and drinking water. After twenty-one days of exposure, they were sacrificed after an overnight fast under diethyl ether as anesthesia. Blood samples were collected by cardiac puncture. Follicle stimulating hormone (FSH), luteinizing hormone (LH), and testosterone levels were determined using enzyme-linked immunosorbent assay (ELISA).
Results: Results showed that insecticides caused an increase in FSH but decrease in LH and testosterone levels significantly when compared to those in control animals at $P<0.05$.
Conclusion: The results indicate that prolonged exposure to common household insecticides used in Nigeria has direct effects on FSH, LH and testosterone levels and may reduce fertility in rats.

Keywords: Insecticides; testosterone; FSH; LH; infertility.

1. INTRODUCTION

Insecticides are substances used to kill, repel or mitigate one or more species of insects. They also include ovicides and larvicides used against insect eggs and larvae, respectively [1]. Insecticides are used in agriculture, healthcare, industry and by individuals. Insecticides have been reported to be a major factor behind the increase in the 20th century's agricultural productivity [2]. Nearly all insecticides have the potential to significantly alter ecosystems; many are toxic to humans and/or animals; some are accumulate in the body and bio-magnify as they pass along the food chain [2].

Insecticides can be classified into two major groups: systemic insecticides, which have residual or long term activity and contact insecticides, which have no residual activity. The mode of action describes how the pesticide kills or inactivates a pest. It provides another way of classifying insecticides [1]. Mode of action can be important in understanding whether an insecticide will be toxic to unrelated species, such as fish, birds and mammals. Insecticides may be repellent or non-repellent. Social insects such as ants cannot detect non-repellents and readily crawl through them. As they return to the nest they take insecticide with them and transfer it to their nestmates. Over time, this eliminates all of the ants including the queen. This is slower than some other methods, but usually completely eradicates the ant colony [3]. Insecticides are distinct from non-insecticidal repellents, which repel but do not kill. The common household insecticides used in Nigeria are systemic insecticides. They include Rambo produced by Gongoin and Co, Mortein produced by Reckitt Benckiser, Raid and Baygon both produced by S.C. Johnson and Co. People use these insecticides without consideration of their adverse effect on health.

Infertility is defined as the inability to achieve pregnancy after 12 months of unprotected intercourse [4]. Male infertility is found in 50% of infertile couples [5]. According to Speroff and Fritz [6], 55% of the reasons for infertility are found to be male-related and 35% to be female-related, while 10% constitutes infertility of unknown origin [6]. Some of the etiologies of declining male fertility can be related to falling androgen levels, decreased sexual activity, alterations in sperm quality, especially, motility, morphology, and DNA integrity [7]. Gonadotropin releasing hormone (GnRH) secreted by the hypothalamus elicits the release of gonadotropins i.e. follicle stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary gland [8]. LH is a glycoprotein that regulates testosterone synthesis by the extratubular Leydig cells. The other gonadotropic hormone, FSH controls spermiocytogenesis and spermiogenesis by affecting both the germinal epithelium and Sertoli cells [9]. The levels of these hormones are under negative feedback control by the gonads [10]. Testosterone is responsible for normal growth, development of male sex organs, and maintenance of secondary sex characteristics. A high intratesticular level of Testosterone is an absolute prerequisite for sperm production, and function. Testosterone improves sperm motility and epididymis function [11]. Failure of pituitary gland to secrete FSH and LH will result in disruption of testicular function leading to infertility [12].

Semen is an organic fluid that contains spermatozoa. It is secreted by the gonads (sexual glands) and other accessory sex organs of male, and can fertilize female ova. In humans, semen contains several components besides spermatozoa: proteolytic and other enzymes as well as fructose which is the major energy source of spermatozoa, and provide a medium through which they can move or "swim" [13]. Male infertility can be assessed through semen

analysis and hormonal profile [14]. The aim of this study is to access the effect of common household insecticides used in Nigeria on male reproductive hormones.

2. MATERIALS AND METHODS

2.1 Collection of Insecticides

Baygon, Raid and Mortein insecticides were purchased from the supermarket while Rambo was purchased from a Pharmacy both in Ibadan, Nigeria. They were kept at room temperature before and during the experiment.

2.2 Experimental Design and Animal Treatment

Thirty male Wistar rats weighing between 220 and 245 g were used for this study. They were acclimatized for seven (7) days to laboratory conditions before the commencement of the experiment. During this period, they were fed *ad libitum* with standard feed and drinking water and were housed in clean cages placed in well-ventilated housing conditions (under humid tropical conditions) throughout the experiment. All the animals received humane care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science and published by the National Institute of Health. They were randomly divided into five groups of six rats each and kept in different rooms. Rats in group 1 were exposed to Rambo insecticide, those in group 2 were exposed to Mortein insecticide, those in group 3 were exposed to Raid insecticide, those in group 4 were exposed to Baygon insecticide while those in group 5 were not exposed to any insecticide and served as the control group. The exposure was done twice a day via inhalation route. Throughout the experiment, they were fed *ad libitum* with standard feed and drinking water. After twenty-one days of exposure, the rats were sacrificed after an overnight fast under diethyl ether as anesthesia. Blood samples were collected via cardiac puncture.

2.3 Determination of Male Reproductive Hormones

The serum levels of FSH, LH, and testosterone were measured by using enzyme-linked immunosorbent assay (ELISA) according to the methods described in Manafa et al. [15].

2.4 Statistical Analysis

Data were subjected to analysis using Analysis of Variance (ANOVA) with the aid of graph pad prism. Data from each parameter was expressed as mean value ± standard error of the mean (SEM). Data were considered to be significantly different at 95% confidence level ($P<0.05$).

3. RESULTS

The results of the effect of insecticides on FSH, LH and testosterone are presented in Figs. 1-3. In the present study, elevated serum levels of FSH were observed in animals exposed to Rambo, Raid and Baygon which was statistically significant when compared with levels in the control group at $P<0.05$ while Mortein had no significant different (Fig. 1). All the insecticides used in this study were observed to have significantly reduced the levels of serum LH when compared with levels in the control group at $P<0.05$ (Fig. 2). According to the result, all the insecticides used in this study significantly decreased the levels of serum testosterone when compared with levels in the control group at $P<0.05$ with Raid having the most significant effect (Fig. 3).

4. DISCUSSION

It is highly important in the investigation of male infertility to consider the levels of reproductive hormones. It has been reported that these hormones have vital roles in male spermatogenesis [16]. It has been reported that the relationship between hormone concentration and parameters of testicular functions are quite variable, and abnormal spermatogenesis sometimes occur concurrently with endocrine abnormalities [17]. FSH, LH and testosterone are prime regulators of germ cell development. The quantitative production of spermatozoa generally requires the presence of FSH, LH and testosterone. FSH acts directly on the seminiferous tubules whereas luteinizing hormone stimulates spermatogenesis indirectly via testosterone [18].

There are several possible mechanisms for the antigonadal actions of organophosphates (a constituent of insecticide); they may exert a direct inhibitory action on the testis; they may affect the pituitary, causing changes in gonadotrophins concentrations and thus subsequent spermatogenic impairment; or they

may change the concentration of neurotransmitter [19]. Antiandrogens can disrupt male differentiation by several mechanisms, including antagonism of receptor binding, or by inhibition of the production, transport, or metabolism of androgens [20]. In the present study, elevated serum levels of FSH were observed in animals exposed to Rambo, Raid and Baygon which was statistically significant when compared with levels in the control group at P<0.05 (Fig. 1). This could probably be due to suppression of feedback inhibition of anterior pituitary [21]. The suppression of feedback inhibition may secondarily increase the secretion of FSH. The results of the present study indicate that these insecticides have a direct effect on pituitary, which led to increase in circulating FSH levels in the blood. FSH has important effects on Sertoli cells. Inhibin and other factors secreted by Sertoli cells cause increase of circulating FSH levels by feedback on pituitary. This corresponds to the findings of Fattahi et al. [21] who reported a significant increase in serum FSH level when animals were exposed to diazinon, an organophosphate insecticide commonly used in Iran. The result of this study is also in agreement with the reports of De Kretser et al. [22] and Babu et al. [23] who found higher concentration of serum FSH with increasing severity of seminiferous epithelial destruction. Subhan et al. [24] proved that increase in FSH levels may reflect decreased testicular activity resulting in an alteration of the normal feedback mechanism between the testes and the hypothalamic pituitary axis, through an impairment of Sertoli cells, and decreased inhibin secretion. Mann et al. [25] found that tubular damage is always accompanied with a rise in serum FSH. Elevation in serum FSH level observed in this study could be an indication that insecticide exposure might have led to tubular damage in the animals. Yanam et al. [26] found in infertile males with abnormal histopathology (Sertoli cell only syndrome, hypo spermatogenesis and spermatid arrest), the mean FSH levels were significantly elevated compared to the control group. In this study however, Mortein had no significant effect on the FSH levels of animals after 21 days of exposure when compared to those in the control group at P<0.05.

All the insecticides used in this study were observed to have significantly reduced the levels of serum LH when compared with levels in the control group at P<0.05 (Fig. 2). This might be due to disruption of the spermatogenic process leading to decline in sperm count and infertility [27,28]. This disruption may have occurred by direct toxic effects of the insecticides on cells and tissue, or it might also occur because of

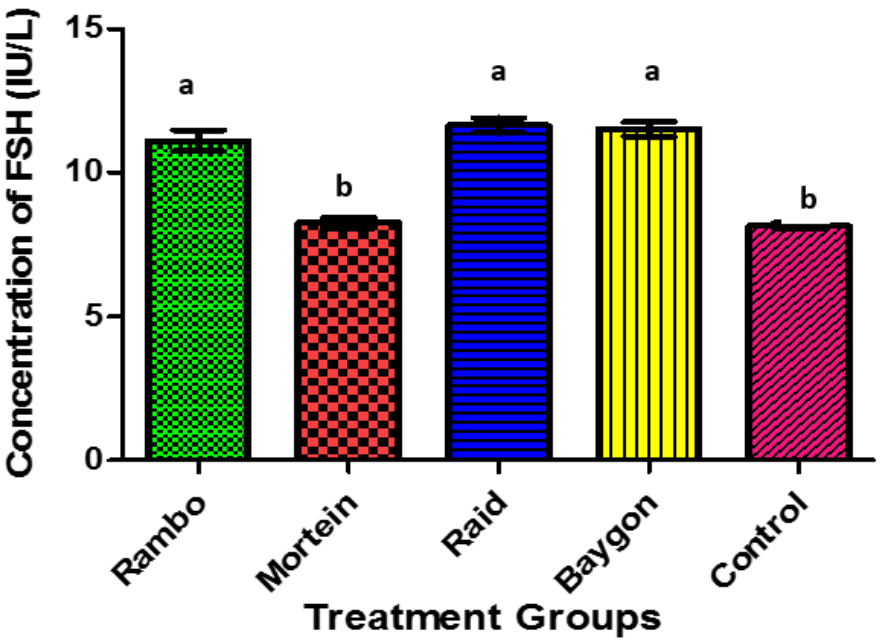

Fig. 1. Effect of household insecticides on the concentration of Follicle Stimulating Hormone (FSH) in animals after 21 days of exposure

Results are presented as mean ± SEM with n = 6. Bars with different letters are significantly different at P<0.05

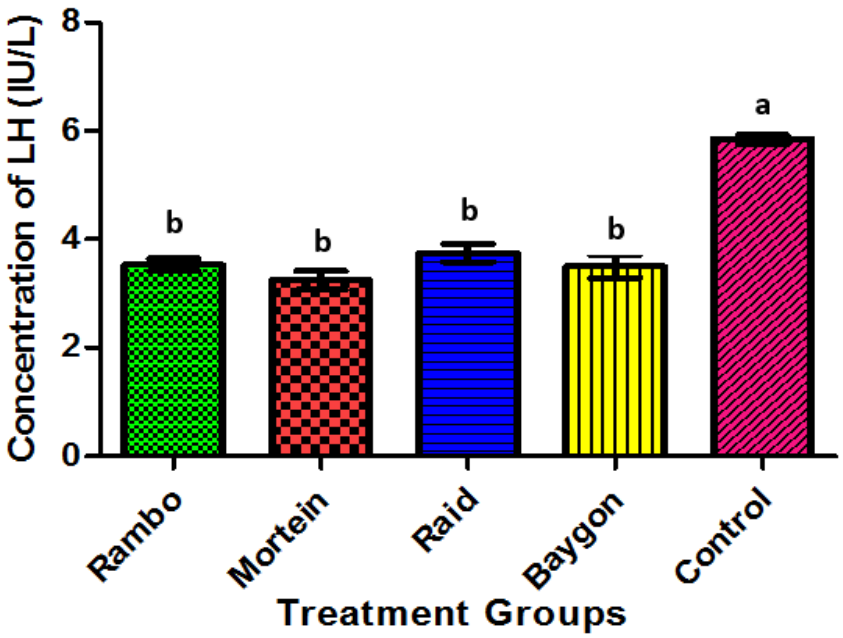

Fig. 2. Effect of household insecticides on the Concentration of Luteinizing Hormone (LH) in animals after 21 days of exposure

Results are presented as mean ± SEM with n = 6. Bars with different letters are significantly different at P<0.05

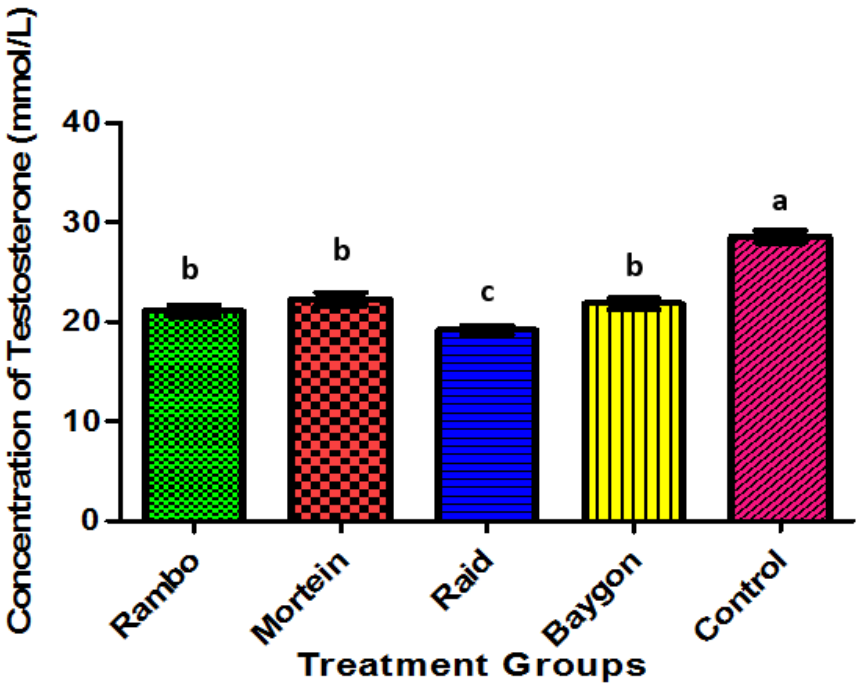

Fig. 3. Effect of household insecticides on the concentration of testosterone in animals after 21 days of exposure

Results are presented as mean ± SEM with n = 6. Bars with different letters are significantly different at P<0.05

imbalanced hormones levels [21]. The insecticides used in this study might have direct effect on testis tissue or by entering into the pituitary gland which could cause decrease in the level of LH. The primary role of LH in the male is to stimulate the production of testosterone by the Leydig cells [29].

Fig. 3 shows the result of the effect of common household insecticides used in Nigeria on testosterone. According to this result, all the insecticides used in this study significantly decreased the levels of serum testosterone when compared with levels in the control group at $P<0.05$ with Raid having the most significant effect. The inhibitory effects of chemicals with estrogenic activities on sex hormone (such as testosterone) secretion have been reported by several other groups [30-32]. Decreased levels of LH and the damage of Leydig cells might account for reduced testosterone production as well as decreased levels of serum testosterone released from testicles observed in this study. Decreased levels of serum testosterone can stimulate the release of gonadotropin-releasing hormone (GnRH) through a negative feedback mechanism. Decreased levels of testosterone may also lead to reduced secretion of seminal fluids from seminal vesicles [33].

Testosterone is a requirement for the differentiation of sex organs and production of sperms [34]. The insecticides used in this study might have declined serum LH and testosterone levels by increasing steroid catabolism and elimination or directly inhibits steroid hormone production [35]. In addition, insecticide has been reported to inhibit steroidogenesis in adrenal cells [33]. Therefore, maintenance of testosterone levels is very critical for spermatogenesis and fertility [36]. Thus, reduced serum testosterone levels arising from exposure of common household insecticides might cause a reduction in spermatogenesis and fertility in animals.

Spermatogenesis in the testes is also regulated by the hypothalamic-pituitary-testicular axis. Gonadotropin (GTH) cells secrete LH and FSH in response to GnRH. GnRH release could also be regulated by testosterone through a negative feedback loop. LH stimulates testosterone production in Leydig cells and FSH stimulates androgen-binding protein (ABP) production in Sertoli cells. ABP binds to testosterone and promotes meiosis of the spermatocytes [33]. The results obtained for serum testosterone which is the prevalent male sex hormone, agrees with the findings of Darbre [37], who reported that air pollution can have serious hormonal effects, as many environmental pollutant chemicals have been shown to possess the ability to interfere in the functioning of the endocrine system and have been termed endocrine disrupting chemicals. Though the exact mechanism on how these hormones are affected by the pollutants remain poorly understood, but it can be similar to that of the ubiquitous chemical bisphenol A which looks and acts like sex hormones thereby binding to the receptors of the sex hormones causing a disruption in the functioning of the endocrine system and resulting in an alteration in sex hormone concentration [32].

Darbandi et al. [38] has reported that the pivotal hormonal regulators of male reproductive functions can be affected by the disruption of the balance between reactive oxygen species production and the antioxidant defense mechanism in the male reproductive system. Uncontrolled generation of reactive oxygen species may directly damage reproductive tissues or can interfere with the normal regulatory mechanisms of the hypothalamic-pituitary gonadal axis and its crosstalk with other endocrine axis, to adversely affect male reproductive functioning, thereby inducing male infertility [38]. From their study, following the generation of reactive oxygen species, the hypothalamic pituitary axis becomes activated and releases cortisol (in humans) in response to stress. These stress hormones, through the cross-talk between the hypothalamic-pituitary gonadal and hypothalamic-pituitary axis, negatively affect luteinizing hormone secretion from the anterior pituitary. Sahinoz et al. [39] has previously reported that insecticide exposure increased generation of free radicals and induced oxidative stress. The adverse effect of common household insecticides used in Nigeria on male sex hormones in this study could also arise from generation of free radical and induction of oxidative stress by the insecticides.

5. CONCLUSION

Insecticides have adversely affected the reproductive hormones in male Wistar rats. The results of this present study indicate that prolonged exposure to common household insecticides used in Nigeria has adverse effects on FSH, LH and testosterone levels and may reduce fertility. Staying in a room fumigated with these insecticides when the effect has not completely subsided should be discouraged.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard written ethical permission has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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